"Collections of accessible region sequences from a particular cell can be cloned to generate a library . . ." Note again the use of the plural "accessible region sequences."

Thus, the Office's statement that the term "library" has not been defined by Applicants is incorrect. Moreover, Applicants' definition of library clearly excludes the cited clones disclosed by Grosveld. Accordingly, the rejection should be withdrawn.

CONCLUSION

Applicants believe that the pending claims are non-obvious over the cited reference for the reasons of record, including those set forth in this Response.

Respectfully submitted,

Date: May 18, 2004

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CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 2

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INTRODUCTION

The usual approach to isolating a recombinant DNA clone encoding a particular gene or mRNA sequence is to screen a recombinant DNA library. As described in Chapter 5, a recombinant DNA library consists of a large number of recombinant DNA clones, each one of which contains a different segment of foreign DNA. Since only a few of the thousands of clones in the library encode the desired nucleic acid sequence, the investigator must devise a procedure for identifying the desired clones. The optimal procedure for isolating the desired clone involves a positive selection for a particular nucleic acid sequence. If the desired gene confers a phenotype that can be selected in bacteria, then only the desired clone will grow under selective conditions, and it can be isolated in a rapid, effortless fashion. However, most eukaryotic genes and even many bacterial sequences do not encode a gene with a selectable function. Clones encoding nonselectable sequences are identified by screening libraries: the desired clone is identified either because it hybridizes to a nucleic acid probe or because it expresses a segment of protein that can be recognized by an antibody.

Screening libraries involves the development of a rapid assay to determine whether a particular clone contains the desired nucleic acid sequence. This assay is used first to identify the recombinant DNA clone in the library and then to purify the clone (see Fig. 6.0.1). Normally, this screening procedure is performed on bacterial colonies containing plasmids or cosmids or on bacteriophage plaques. To test a large number of clones at one time, the library is spread out on agarose plates (*UNIT 6.1*), then the clones are transferred to filter membranes (*UNIT 6.2*). The clones can be simultaneously hybridized to a particular probe (*UNITS 6.3* and *6.4*) or bound to an antibody (*UNIT 6.7*). When the desired clone is first identified, it is usually found among many undesirable clones; an important feature of library screening is the isolation of the desired clones (*UNITS 6.5* and *6.6*). Another method for identifying the desired clone involves hybrid selection (*UNIT 6.8*), a procedure in which the clone is used to select its mRNA. This mRNA is characterized by its translation into the desired protein.

To screen a DNA library, one must first devise the screening procedure. The next important choice is the selection of a recombinant DNA library. When choosing which library to screen the investigator should consider whether he or she wants to isolate clones encoding the gene or the mRNA sequence. cDNA clones will encode the mRNA

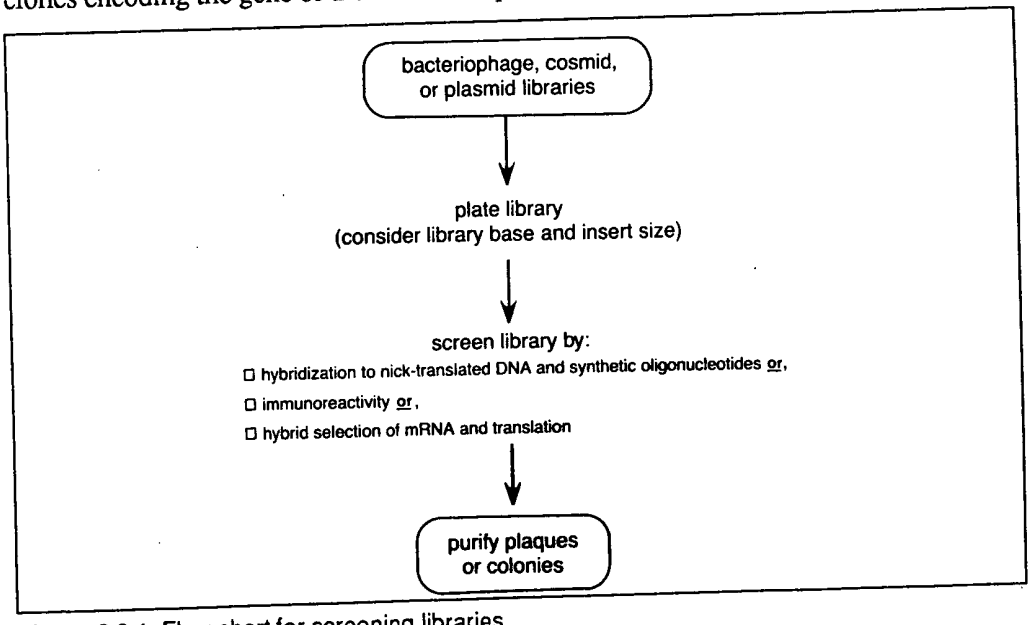

Figure 6.0.1 Flow chart for screening libraries.

Methods in Enzymology

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Guide to Molecular Cloning Techniques

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plaques *in situ* to replace those that have been removed during the preparation of filters or to increase subsequent hybridization signals.

1. Prepare an overnight culture of plating bacteria (this volume [13 or 17]). You will need 400 ml of culture for 20 filters.
2. Collect bacteria by centrifugation and resuspend them in an equal volume of fresh LB + 10 m*M* $MgSO_4$. General methods for handling λ are found in this volume [13].
3. Label the filters and mark them asymmetrically, with a black ballpoint pen, on the side that will be in contact with the plaques.
4. Dip the filters in the bacterial cell suspension and allow them to air dry briefly.
5. Lay the filters on the surface of the plates containing plaques. Transfer the orientation marks to the agar plate. The techniques in step 4 of the alternate procedure can be employed.
6. Prepare additional filter copies, if desired. Be sure to transfer orientation marks from agar to filter. A light box is a useful aid here.
7. Lay copy filters, phage plaque side up, on fresh LB + Mg^{2+} plates and incubate, inverted, at 37° overnight.

During the overnight growth at 37° the plaques infect the growing *E. coli*, leading to a substantial amplification of phage DNA. After this amplification, it is usually not necessary to hybridize two sets of filters to avoid false positives.

8. Remove the filters from the plates, air dry for at least an hour and process filters as in step 16, omitting the 10% SDS treatment as described above. Store plates, inverted, sealed in Parafilm at 4°. The filters are ready for prehybridization (this volume [45]).

[45] Screening Colonies or Plaques with Radioactive Nucleic Acid Probes

By GEOFFREY M. WAHL and SHELBY L. BERGER

Colony or plaque hybridization is a technique for screening replicated material *in situ* on filters with labeled probes.[1–5] The probes most com-

[1] M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72,** 3961 (1975).
[2] M. Grunstein and J. Wallis, this series, Vol. 68, p. 379.
[3] W. D. Benton and R. W. Davis, *Science* **196,** 180 (1978).
[4] D. Hanahan and M. Meselson, *Gene* **10,** 63 (1980).
[5] D. Hanahan and M. Meselson, this series, Vol. 100, p. 333.

monly used are nucleic acids or antibodies. Here we will describe techniques for using nucleic acids to analyze libraries generated in either phages or plasmids. The use of antibodies for screening libraries can also be found in this volume [50, 51].

A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants, only one or a few of which are of immediate interest to the investigator. The desired clone is located by performing the following steps: (1) transfected bacteria or phage are grown on master plates (or filters) and replica plated; (2) the original plates called *master plates* are preserved while the replicas, hereafter called *filters,* are processed; (3) phage are disrupted or bacteria are lysed *in situ* on filters; (4) DNA is bound to the filter while RNA is hydrolyzed; (5) the resulting partially denatured DNA is hybridized to sequences able to bind specifically to the desired insertions. (6) Because the configuration of DNA on the filter replicas matches the configuration of live bacteria or phage on the master plates, DNA on replicas which binds to the probe (so-called positive signals) can direct the investigator to the bacterial colony or phage plaque from which the DNA was derived; (7) the positive colony or plaque is then purified and grown in quantity for further analysis.

Chapters [44] and [18] describe steps 1–4 for plasmid or λ libraries and cosmid libraries, respectively. Here we will focus on steps 5–7.

Colony hybridization is a rapid but inexact procedure aimed at calling attention to clones worthy of serious consideration. Falsely positive clones are therefore not uncommon. To some extent these can be reduced by the following: (1) use both negative control filters and, if possible, positive control filters; (2) screen duplicate filters of each master plate; and (3) prepare probes carefully.

To satisfy the requirements of point 1, it is advisable to include clones containing the vector without an insert or containing an irrelevant insert. The latter is particularly important when fragments bearing homopolymer "tails," usually composed of dG on one strand and dC on the other, are screened; the GC-rich regions on either end of the insert can hybridize to GC-rich probes and cause spurious positive signals. Thus, the use of known negative recombinants acts as a means for detecting unwanted cross-hybridization of the probe to vector and host DNA (which are also present) and also serves to establish the intensity of a background signal, one that should be ignored. Since intensities are relative, a genuine positive signal is needed for comparison. If there are no known positive clones, one can always clone the probe itself and create a positive recombinant. Such engineered positive colonies or plaques are rarely perfect